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Dr. H. Neal Bramson

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19 ABSTRACT (Continue on reverse if necessary and identify by block number)

We have taken a modular approach to study peptide interactions with proteins as a model for protein-protein interactions. Two basic approaches were applied to this problem, and peptides were designed to bind either a specific sequence of double stranded DNA or the pp56$^{lck}$ tyrosine protein kinase. This protein kinase likely is the cytoplasmic effector of CD4, a transmembrane receptor whose activation *in vivo* is associated with an increase in cytoplasmic free calcium. Peptides have been synthesized which bind to each of these macromolecules with $K_{Ds}$ near 2mM. In addition to binding pp56$^{lck}$ tightly, one peptide has been found to stimulate the activity of this enzyme up to 20-fold. We have shown that ionic interactions are important for this activation, and that these peptides alter the substrate specificity of this enzyme. Due to our greater success with the protein kinase project, we have recently been concentrating our efforts on this project. pp56$^{lck}$ binding peptides are being studied to enable modelling signal transduction across membranes. In addition, our finding that synthetic peptides can recognize proteins within biological mixtures has led us to design a series of peptides bearing photoaffinity labels. These photoaffinity label bearing peptides model portions of proteins known from molecular biology studies to mediate interactions with unknown proteins. We are currently developing methods utilizing the photoaffinity peptides to allow the identification of these unknown proteins.

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**FINAL REPORT** **DATE:** 16 April 1990

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**PRINCIPAL INVESTIGATOR:** H. Neal Bramson

**CO-INVESTIGATORS:** Lisa Regan and Chris Sommers

**CONTRACTOR:** The University of Rochester

**CONTRACT TITLE:** A Modular Approach to Protein Design

**START DATE:** 1 February 1988

**RESEARCH OBJECTIVES:**

We are investigating protein-protein interactions utilizing peptides that reproduce segments of protein structures to simplify this problem. In this way DNA-protein interactions have been studied through incorporating the helix-turn-helix of the DNA binding *lac* repressor into ribonuclease A to transform this protein into one which binds double stranded DNA. Similarly, in order to study protein-protein interactions a peptide incorporating a region of the CD4 receptor has been synthesized and shown to modulate the activity of pp56$^{lck}$, the physiological ligand of the CD4 receptor, over a range almost 100-fold. The long term goal of the pp56$^{lck}$ work is to attach a regulatory module to this activating peptide and make this activation responsive to small molecules. Finally, we have recently been developing methods to use photoaffinity label containing synthetic peptides that reproduce segments of proteins which alone seem to interact with other proteins as carriers to selectively modify those proteins. By incorporating groups such as biotin into these photoaffinity peptides we hope to facilitate the retrieval of these probes following their modification of protein targets. Due to efficient incorporations of such labels into protein targets, this technology will be generally useful for elucidating protein-protein interactions.

**PROGRESS:**

<u>DNA BINDING MODULES</u>

The sequence of the first DNA binding module we synthesized is:

10
Met-Lys-Pro-Val-Thr-Leu-Tyr-Glu-Gln-Ala-Glu-Gln-Ala-Gly-Val-Ser-Tyr-Val-Gln-
20 30
Val-Ser-Arg-Gln-Val-Asn-Ala-Ala-Ser-Lys-Phe-Glu-Arg-Gln-His-Met-Asp-Ser

PEPTIDE 1

Peptide 1 is comprised of *lac* repressor residues 1-28 fused to ribonuclease residues 7-15 with several amino acid residue changes made to increase the stability of peptide structure. As expected, peptide 1 bound tightly to the ribonuclease S protein ($K_D$=3μM). A CD analysis of peptide 1 in the presence and absence of the S protein indicated that the complexed peptides are substantially more helical than the free peptides. It appeared therefore that at least some of the desired peptide structure (such as the *lac* repressor helix-turn-helix) was present in the complex. However, when peptide 1 and ribonuclease S protein were incubated together with a 180bp PUC18 fragment which includes the *lac* operator, no sequence specific DNA binding was detected by gel shift assays and

DNAase footprinting. At concentrations above 50μM the complex did bind to the DNA in a nonspecific fashion which was competed off with calf thymus DNA or high concentrations of salt.

We therefore synthesized two peptide modules that retained more alanine residues to stabilize the helices and additional *lac* repressor residues:

10
Met-Lys-Lys-Val-Thr-Leu-Tyr-Glu-Ala-Ala-Glu-Ala-Ala-Gly-Val-Ser-Tyr-Gln-Thr-
20 30
Val-Ser-Arg-Lys-Val-Ala-Ala-Ala-Ala-Lys-Phe-Glu-Arg-Gln-His-Met-Asp-Ser

**Peptide 2**

and peptide 3 which contains an asparagine, such as is found in the *lac* repressor, at residue 25 in place of alanine. Each peptide binds the ribonuclease S protein approximately as well as does peptide 1. However, as part of the complex with the S protein, peptides 2 and 3 each bind to a 140bp fragment which contains the *lac* operator at least 10-fold more tightly than the complex containing peptide 1. Even at concentrations of about 5μM of complex DNA binding is efficient and not inhibited by 0.25M concentrations of salt. Higher salt concentrations inhibited not only this interaction but also substantially decreased DNA binding by the native *lac* repressor. Complexes containing peptides 2 and 3 also bind tightly to sequences that do not contain the *lac* operator, including other PUC18 fragments and calf thymus DNA. Although several methods were investigated, we never were able to demonstrate sequence specific DNA binding by either peptide. Because all helix-turn-helix proteins known seem to bind DNA as dimers, we attempted to investigate the effect of dimerizing peptides on their DNA binding activities.

To facilitate comparisons between specific and nonspecific DNA binding we synthesized a palindromic oligonucleotide which self anneals to form a strong *lac* repressor binding site:

TTAATTGTGAGCGCTCACAATT

We also synthesized a 51 amino acid residue fragment of the *lac* repressor referred to as the *lac* headpiece to characterize peptide binding to the oligonucleotide. The *lac* headpiece has been shown in a number of laboratories to bind to sequences similar to the above oligonucleotide. We used filter binding assays to measure the association of oligonucleotide and the headpiece. The effect of dimerization, which might be useful for increasing the DNA binding affinities of peptide modules, on *lac* headpiece DNA binding, was also investigated. We found that monomeric *lac* headpiece binding to the above oligonucleotide is characterized by a $K_D$ of about 3μM and that when this peptide is dimerized through oxidation of a cysteine residue included on the carboxy terminus of the headpiece this binding interaction was not significantly tighter. We therefore synthesized a peptide known to form tetramers and attached this to the *lac* headpiece, but this also did not alter the binding of this headpiece to DNA. As we still have no way to increase peptide-DNA binding to the extent that sequence specific binding can be seen.

PROTEIN-PROTEIN INTERACTIONS

Cytoplasmic tyrosine protein kinases of the *src* family are protooncogene products that are thought likely to play crucial roles in signal transduction through cellular receptors. One of these enzymes, $pp56^{lck}$, is associated with the transmembrane receptor CD4 in $CD4^+$ T cells. There is now substantial evidence that the 38 amino acid residue cytoplasmic domain of this receptor regulates the

activity of the protein kinase through protein-protein interactions. The goal of this project is to elucidate the mechanism for this regulation to enable the synthesis of peptide models which reversibly regulate enzymic activities.

Our results are summarized as follows, and a copy of a paper in press describing these results is included in this mailing. The peptide:

10
Acetyl-Tyr-Val-Arg-Cys-Arg-His-Gln-Gln-Arg-Gln-Ala-Ala-Arg-Met-Ser-Gln-Ile-
20 30
Lys-Arg-Leu-Leu-Ser-Glu-Lys-Lys-Thr-Cys-Gln-Cys-Pro-His-Arg-Met

MODULATOR 1

reproduces most of the cytoplasmic domain of the CD4 receptor. When $pp56^{lck}$ catalyzed phosphorylation of peptide substrates is measured in the presence and absence of modulator 1, we found that modulator 1 changed the substrate specificity of this protein kinase up to about 100-fold. The phosphorylation of some peptides is increased more than 20-fold in the presence of modulator 1, while the phosphorylation of other peptides is inhibited. This result implies that specific receptors customize the activities of their associated protein kinases, which would explain how the same protein kinase can play varying biological roles depending on cell type. In addition to this customization, receptors also influence what substrates the protein kinase encounters. We are continuing to investigate these possibilities.

DETECTION OF PROTEIN-PROTEIN INTERACTIONS UTILIZING PHOTOAFFINITY PEPTIDES

We have succeeded in detecting a protein that normally interacts with the $p60^{c\text{-}src}$ protein kinase using a peptide which reproduces the N-terminal portion of this protein kinase. The peptide:

Myr-Gly-Ser-Ser-Lys-Ser-Lys-Pro-Lys-Asp-Pro-Ser-Gln-Arg-Arg-Arg-BPh-[$^{125}$I]Tyr

**[$^{125}$I]PHOTOAFFINITY PEPTIDE**

reproduces the myristic acid (Myr) and N-terminal 15 amino acid residues of $p60^{c\text{-}src}$ plus the photoaffinity label *p*-benzoyl L-phenylalanine (BPh) and a tyrosine residue. When placed in the presence of platelet cell membranes, a very rich source of $p60^{c\text{-}src}$, and exposed to strong light composed of wavelengths 320 nm and above, 100nM [$^{125}$]photoaffinity peptide alone specifically labels a 90 kD platelet *src* receptor. This modification is prevented by an excess of unlabeled peptide, but not by the addition of 0.01 mg/ml polylysine. To further demonstrate the specificity of the iodinated photoaffinity peptide for the 90 kD protein, photolysis was also performed in the presence of 125 μM Myristyl-Gly-Ser-Ser-Lys-Ser-Lys-Pro-Lys-Asp-Pro-Ser-Tyr (lane 1), which contains 4 fewer $p60^{src}$ residues at its C-terminus than the photoaffinity peptide and has a 4000-fold higher $K_D$ for binding to the putative *src* receptor than the sequence contained in the photoaffinity peptide. Thus, this truncated peptide would not be expected to inhibit the binding of 1000-fold lower photoaffinity peptide concentrations to the platelet *src* receptor, and consistent with expectations, *src* receptor is labeled by the photoaffinity peptide despite the presence of truncated peptide. The labeling of the *src* receptor in the presence of truncated peptide but not cold photoaffinity peptide demonstrates high affinity interactions between the platelet *src* receptor and the photoaffinity peptide, allowing photoaffinity labeling to be a useful technique for isolating this receptor.

Because we have encountered problems in synthesizing iodinated peptides containing the benzophenone photoaffinity reagent, peptides containing other

radiolabels are now being synthesized. In addition, we have also begun to synthesize photoaffinity label containing peptides that reproduce the sequences of several other protein kinases in order to detect what proteins these enzymes associate with. We plan to continue exploring the use of photoaffinity label containing peptides to identify protein-protein interactions, and will locate sites for protein-peptide interactions through protein sequencing.

**PUBLICATIONS**

1. Bramson, H. N., Casnellie, J. E., Nachod, H., Regan, L. M., and Sommers, C. "Synthetic fragments of the CD4 receptor cytoplasmic domain and large polycations alter the activities of the pp56$^{lck}$ tyrosine protein kinase." J. Biol. Chem., in press.

**TRAINING ACTIVITIES**: One graduate student has been assisting in this work.

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